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The association of some cytokines, ceruloplasmin oxidase activity and copper concentration in male infertile patients

Suha Hanoon Hasan [1] Hathama Razooki Hasan [2] Alice K. Melconian [3]
[1,2,3] *Department of Chemistry, College of science, University of Baghdad, Baghdad, Iraq*

Abstract

Various Ceruloplasmin(CP) has been described as a moon lighting protein due to its many and varied activites, it carries up to 90% of plasma copper.Cytokines are groups of soluble protein which act as immunomodulatory elements in male gonad. The aim of the present study was to highlight the relevance of some cytokine in male infertility as well as to investigate if they have any relationship to CP as antioxidants. The current study included three groups of Iraqi male, 70 infertile patients(35 with oligospermia, 35 with azoospermia,and 40 fertile healthy as a control group. The measured parameters included serum and seminal plasma ceruloplasmin oxidase activity,the concentration as copper,Interleukin-17(IL-17),Interleukin-10(IL-10) ,and Tumor necrosis factor alpha(TNF-α). The results in comparison with that of the control groups showed:-

1). A significant decrease ($p<0.05$) in seminal plasma Cp oxidase activity in azoospermia group, meanwhile the specific activity revealed highly significant decrease ($p<0.001$) in seminal plasma of both studied group. 2). A highly significant decrease($p<0.001$) in serum copper concentration of the patients groups.3). A highly significant increase ($p<0.001$) in serum concentration of TNF-α in both of the studied patient groups,while there comparison of this concentration in the seminal plasma samples of the same groups showed significant decrease($p<0.05$) only in oligospermia group as well as significant differences($p<0.05$)between both of the studied groups . 4). A highly significant decrease ($p<0.001$) in serum concentration of IL-17 of azoospermia group and highly significant difference($p<0.001$) between the two studied groups,while in seminal plasma showed highly significant increase($p<0.001$) of both of the studied groups with the control on one hand and highly significant differences($p<0.001$) between the two studied groups on the other hand. 5). A significant decrease ($p<0.05$) in seminal plasma concentration of IL-10 of both of the studied groups.

Correlation analysis between serum and seminal plasma of all the parameters showed:- 1). Negative correlation between serum and seminal plasma of control group of Cp oxidase activity and specific activity

2). positive correlation between serum and seminal plasma of azoospermia group of TNF concentration.

Correlation analysis among all the parameters showed:- :- 1). Strong positive correlation between Cp oxidase and TNF-α in serum of control group.

2). Positive correlation between IL-17 and TNF-α in serum of oligospermia group.

3).Negative correlation between IL-17 and SP cp oxidase activity in serum of control group. 4). Negative correlation between Cp oxidase activity and IL-10 in serum of azoospermia group.

5). Negative correlation between IL-10 and SP CP oxidase activity in serum of azoospermia group.

6).Positive correlation between IL-10 and IL-17 in serum of control group. 7). Positive correlation between IL-10 and IL-17 in seminal plasma of oligospermia group. 8).Negative correlation between IL-10 and copper concentration in seminal plasma of azoospermia group. Seminal plasma Cp oxidase&specific activity, TNF-α and IL-17 were negatively correlate with sperm motility within non significant value.

Key words: Male infertility,seminal plasma, Ceruloplasmin oxidase activity,IL10,IL17,TNF-α.

INTRODUCTION

Globally,the incidence of infertility is estimated to be about 13-18%(1),some reports mentioned that approximately.50% of infertile couple is due to male factor (2) while others state that infertile prevalence is increasing in all societies and it may reach up to one forth in all married couples(3).

Male infertility factor can be classified as a change in sperm concentration, morphology, and motility (4)which may involve the deficiency or excess of various factors important for spermatogenesis(2). Therefore seminal analysis is the first step in assessing male fertility and the scores of movement, numbers and abnormality are the interesting sub topics in male semen examination(5) .

Oligospermia and azoospermia are the main causes of male infertility. Oligozoospermia is the pathological depression in accepted number of sperms in semen,and it is responsible for 90% of infertility in man(6,7).While Azoospermia is the condition which is characterized by the absence of semen sperm(8) ,affects about 1% of the male population (9) and may be seen in up to 20% of situation of male infertility(10) . Research findings demonstrate that not all male with normal seminal analysis are fertile. The hidden factor is the oxidative stress that is characterized as important and probable cause of idiopathic male infertility(11).Idiopathic male infertility is a part from the conventional cause for male infertility as in case of varicocele, Cryptorchidism, infection, obstructive lesions, cystic fibrosis, trauma and tumors(12).

Although it is a matter of continuing debate there is still only a little informational about relevant factors which might negatively influence semen quality(13).

Semen is a complex body fluid containing a mixture of spermatozoa suspended in secretion from the testes and epididymis which are mixed with secretions from other accessory sex glands such as the prostate and seminal vesicles at the time of ejaculation (14).

Oxidative stress(OS) caused by accumulated reactive oxygen species (ROS) is closely involved in a variety of pathological processes (15).

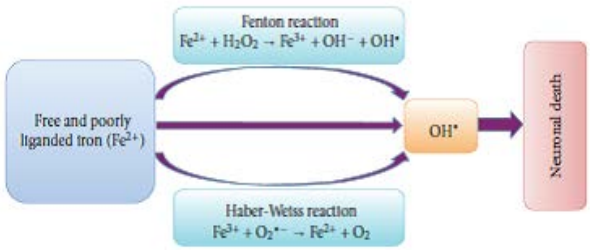
The ROS has been reported to play an important role in the complex biochemical cascade of the spermatozoa and then require antioxidants protection at sites of gamete product maturation and storage (15).

The OS is a result of imbalance between ROS and antioxidant that lead to sperm damage,deformity and gradually male infertility (16),this is due to the susceptibility of mammalian sperm membrane to lipid peroxidation as a result of the high amount of poly unsaturated fatty acid in the phospholipids of their membranes(17).

One of the important antioxidant in the plasma is ceruloplasmin which is a glycoprotein that is considered as one of the acute phase protein.. Furthermore it is a multifunction plasma protein that has amino oxidase as well as ferroxidase activity and it carries up to 90% of plasma copper ,by such binding it prevents copper from involvement in free radical formation(18).

Excess or decrease of copper may lead to abnormal or defect spermatogenasis and oxidative damage to testicular tissue and spermatozoa which lead to infertility(19).This is due to the role

that is played by this transition metal together with iron in free radical production via reactions fenton and haber- weiss equation(19):



Human semen is composed of various cells spermatozoa epithelial cell,leukcocytes as well as seminal plasma (SP) .SP contains protein and non protein products derived from sertoli cell,epididymis,seminal vesicle and prostate(20).Among these products are the cytokines .

Cytokines are groups of soluble protein(21) they act as immunomodulatory elements in male gonad (22).They regulate inflammatory and immune response and help in normal physiological function by acting as a growth and differentiation factor that help in normal physiological function(23).

Earlier studies have shown that seminal fluid contains TNF-α ,IL-17 ,IL-10 and other cytokines(23,24).

IL-17 is a pro-inflammatory cytokine that is recently discovered and secreted by activated memory CD4 of T-cell. It induce stromal cells to secrete IL6,8 and promote macrophage to release IL10 and TNF-α(25).While IL-10 is associated with pro inflammatory response, where it promotes the suppression of immune response through it pleiotropic effect (26).

On the other hand Tumor necrosis factor (TNF-α) has different function than other cytokines, it acts on androgenic receptor to regulate **testosterone** activity(27), hence it promotes cell survival through reducing the **testosterone** production and regulate the proportion of this hormone in parts of testis(27).This cytokines also,was reported to have direct effect on sperm motility(23).

In recent years ,a focus of research has been the discovery of relationship between immunity and infertility (28,29).Therefore the aim of the present study was to highlight the relevance of some cytokines in male infertility as well as to investigate their relationships to CP as antioxidants protein in these patients.

MATERIALS AND METHODS

Sampling

Serum and semen samples were collected from 110 male attending Kamal –AL-samerae hospital, including 70 infertile patients with age ranged (22-50) years compared to 40 fertile males as a control with age range compatable to that of the patients. The patients were evaluated by full medical history to exclude any existing systemic diseases that may affect the studies parameters (diabetes,hypertensive,liver disease, renal and cardiac disease).The patients received no antibiotic treatment within one week and were non smoker and non alcoholic, otherwise the patient was excluded from this study. Semen analysis was determined according to WHO criteria (30).The semen samples were collected from all study individuals(110 male) who had been abstinent for 3 to 5 days before the semen collection,liquefaction of semen occurs after a period of 30 min. Semen quality was evaluated macroscopically and by using microscopic examination.The routine seminal analysis was carried out estimating sperm motility, count , morphology, and viability (30).The study protocol has been approved by the ethics committee of Baghdad university /college of science.

The samples were classified depending on seminal fluid analysis according to the WHO criteria(2010)(30) into three groups:-

1-fertile(control group)(n=40) sperm count > 20 million /ml,progressive motility > 50%.

2 – infertile(oligospermia)(n=35) sperm count < 20 million/ml,progressive motility <40% and sperm morphology >50%.

3-infertile (azoospermia)(n=35) sperm count 0%. No sperm in ejaculate. The study protocol approved by the ethics committee.

Blood Samples

Five millilitres of venous blood samples were collected by using disposable needle and plastic syringes from each patient and control.The samples were transferred into clean plain tube,the blood was left at room temperature for 15 min. The serum was separated by centrifugation at (3000 x g) for 10 minutes. The obtained serum was stored at (-20) ºC until being used for subsequent analysis.

Seminal plasma samples

The semen samples were obtained by masturbation into a sterile container after abstinence for 3–4 days and examined within 30 min after liquefaction at $37C^O$. Sperm parameters. After complete liquification ,semen volume, pH,liquefaction time, appearance, density and motility were analyzed according to the method described by WHO (2010). the semen sample was centrifuged at 3,000 rpm for 10 min to pellet sperm. SP was separated and stored at $85C^O$ until analysis.

Determination of CP oxidase activity and specific activity

The CP oxidase activity was measured by using modified Rice method (31). The method based on that CP catalyzes the oxidation of aromatic amines P-Phenylenediamine(used as substrate) to give a blue-violet color that is measured spectrophotometrically at wave length 540 nm.

The Enzyme unit(U) was defined as the amount of a particular enzyme that catalyzes the conversion of one micro mol of substrate per minute. The specific activity of the enzyme was expressed as the enzyme activity per gram of protein

Determination of copper concentration

The copper concentration in serum and seminal plasma were measured by using Flame atomic absorption spectrometry with air-acetylene flame and hollow cathode lamp at wave length 325 nm(32).

Determination of IL17,IL10,TNF-α concentration

The concentration of IL17,IL10 and TNF-α in serum and seminal plasma were determined by quantitative Enzyme-Linked Immunosorbent Assay(ELISA).(GE Health care life sciences(Buckinghamshire,UK) and R&D Systems (Minneapolis,USA) respectively,according to the manufactures instructions.

Statistical analysis

Statistic was performed with SPSS(version 23) software program. The results were expressed as mean±SD .The statistical test used were ,one way ANOVA followed by an LSD test. The correlation coefficient(r) between parameters was determined by analyzing for linear regression.Multiple range test at $p<0.05$ was accepted as significant,and as highly significant when $p<0.001$.

RESULTS AND DISSCUSION

The oxidase activity of CP in serum and seminal plasma were measured as described in the material and method section and the results are presented in Table (1).

It is clear from the results that no significant difference was found when this activity in serum was compared between oligospermia group and the control, while a non significant increase $P>0.05$) in azoospermia was observed.As well as the specific activity revealed no significant differences between the two studied groups and the control .On the other hand a significant decrease ($p<0.05$) in seminal plasma of azoospermia group as compared with that of control group was observed.

The specific activity of Cp oxidase showed highly significant decrease in seminal plasma of both studied groups (P <0.001) as compared with that of control group were noted. It is important to note that cp oxidase and its specific activity were correlated adversely with progressive motility in both fertile and infertile (oligospermia) groups . No significant correlation was found between serum or semen cp oxidase and semen quality

To our knowledge,no study has been reported in the literature that deal with the measurement of ceruloplasmin oxidase activity in male infertility ,but there are several studies carried out in other disease such as ,in Poland a study of Zowczak *et al* (18) which measured the oxidase activity of ceruloplasmin and concentration of copper in serum of cancer patients. Other study in Thailand also focused on the activity of ceruloplasmin oxidase as a biomarker of lead exposure (Leelakunakora *et al*)(33) some of these studies were done by Akalin *et al* (34) in Turkey,which conducted on serum copper and ceruloplasmin concentration with sperm quality in merino rams.Another study by Ranganathan *et al* (35) in Florida , in which the serum ceruloplasmin protein expression and activity increase in iron-deficient rats and further enhanced by higher dietary copper intake.Finally,in Iraq ,other study of ceruloplasmin oxidase activity and C-Reactive protein in sera of patient with diabetes mellitus(36).

Ceruloplasmin(cp) is an ancient multi copper oxidase and it has been described as a moon lighting protein due to its many and various activites ,antioxidant function is related to its ferroxidase activity as well as glutathione ,on the other hand,prooxidant function include amineoxidase and NO oxidase(37). .

The results revealed no significant differences in serum cp oxidase activity neither in its specific activity for the oligospermia group and this may be due to the balance between its expression and activity.

The possible explanation for the non significant elevation of the cp oxidase activity in serum of azoospermia group may be related to the induction of cp activity during infection and inflammation is probably the result of cytokine and hormonal stimulation of cp expression. However,increased cp activity during dietary iron deficiency(35). .

The decline in cp oxidase that was observed in the seminal plasma of infertile men may be due to dietary copper deficiency since liver synthesizes cp, low copper is an important observation suggesting the copper pool for cp synthesis may be limited. The study of (Leelakunakora *et al*)(33) demonstrated that cp containing alpha-2 globulin in serum can serve as a high potential biomarker for lead exposure,cu on the cp molecule has been shown to be required for the oxidase activity,pb may then replace cu on the cp molecule and lead to the reduction of its oxidase activity. The result in Table(1) shows that Cp oxidase activity were higher in seminal plasma of fertile than the infertile,and the differences was statistically significant(p p<0.05) only in case of azoospermia group as characterized by the lack of sperm number,motility or may be related to the defect of regulation of its activity and production.Data obtained from Akahin et al study suggested that seminal plasma cp may be independent from that of blood cp from liver origin,thus sertoli cells secrete cp-like protein(testicular cp)that is immunologically similar to serum cp(34).The decline in specific activity of cp oxidase in seminal plasma may be due to the decrease in the synthesis of protein as the specific activity depend on both activity and protein

The results of copper measurement in sera and seminal plasma of the studied groups are presented in Table(2)

Table (1) Mean value of ceruloplasmin oxidase activity(U/L)& Specific activity(U/g) in sera and seminal plasma of control and patient group.

Groups	Samples N0.	Age(years) (Mean±SD)	Ceruloplasmin oxidase activity(U/L)Mean±SD		Specific activity(U/g) Mean±SD	
			Serum	Seminal plasma	Serum	Seminal plasma
Control	36	32.37±8.258 range (20-48)	45.6 ±14.5	8.03 ±3.63	6.30 ±2.38	2. 72±1.29
Oligospermia	33	33.97±8.090 range(22-46)	43.1 ±13.6 P value 0.56 a 0.09 b	6.82 ±3.19 p.value 0.08 a 0.43 b	5.91 ±1.83 p.value 0.65a 0.21 b	±0.55^{**a} 65.1 p.value 0.000a 0.21 b
Azoospermia	33	32.7±9.096 range (20_50)	50.0±20.5 P value 0.23 a	6.14 ±3.08^{a*} p.value 0.01 a	6.80 ±2.20 p.value 0.24a	1. 20±0.66^{**a} p.value 0.000 a

a* Significant difference in comparison to control at (p≤0.05) **a**** high Significant difference in comparison to control at (p≤0.001). .
b refers to difference between two patients group : difference in comparison to control a refers to

Table (2) Mean Values of copper conc. (µg/ml) in sera and seminal plasma of control and infertile groups

Groups	Sampls no.	Age (years) (mean±SD)	Copper conc (Mean ±SD) Serum × 10^2	Seminal plasma × 10^2
Control	34	32.97±8.25 rang (20-48)	17.9 ±6.1	2.6 ±1.0
Oligospermia	28	33.97±8.09 range (22-46)	^{a**} 11.1±3.1 p.value 0.000 a 0.16 b	2.9 ±1.1 P.value 0.56 a 0.87 b
Azoospermia	26	32.7±9.9 range (20-50)	9.1 ±4.0 ^{a**} p.value 0.000 a	2.8±1.0 P.value 0.64 a

a** high Significant difference in comparison to control at (p≤0.001).

Table(3) Mean Values of Tumor necrosis factor concentration in sera and seminal plasma samples of control and patients groups .

Groups	Samples N0.	Age(years) (Mean±SD	mean±SD) TNF ng/l (Serum	Seminal plasma
Control	25	29.4±4.12 range (20-48)	51.3±15.43	64.4±15.65
Oligospermia	20	35.2±6.90 range(22-46)	a** 66.25±9.22 P value 0.001a 0.66 b 0	a* b** .65±16.7452 P value 0.006 a
Azoospermia	20	31.3±7.52 range (20_50)	a** 64.35 ±7.69 Pvalue 0.000 a	b** .8 ±7.165 P value 0.64 a P value 0.001 b

a* Significant difference in comparison to control at (p≤0.05) **a**** high Significant difference in comparison to control at (p≤0.001). **b****Significant difference in comparison between two patients groups at (p≤0.001).

It is obvious from these results a higher significant decrease($p<0.001$) is presented in this element concentration in serum between both patient groups and the control,while the comparison of this concentration in the seminal plasma samples of the same groups showed no significant differences. No correlation was observed between cu concentration and semen quality.

These results were in a good agreement with Akinloye et al(38) who found no significant difference in seminal plasma Cu level among the three groups of subjects in Nigerian men ,meantime disagree with their results in serum, also the results disagree with the study of Wong et al(39)in the same country which showed that the increase in [Cu^{+2}] in seminal plasma is correlated with reduced sperm motility and postulated that cu concentration could not establish difference between fertile and subfertile male in serum and semen. Also agreed with the study of Valsa et al(40) concluded that the cu level did not correlate with the studied semen parameters.

The results disagreed with the study of Eidi et al (41) who showed that the copper is highly toxic metal for sperm in Iranian male and concluded that seminal plasma copper concentration in oligospermia and azoospermia groups were significantly higher than normal group.

The possible causes of this differences may be due to poor nutritional intake of cu –rich food ,also environmental variation is implicated.

Approximately 90% of the Cu in the blood serum is incorporated into ceruloplasmin, which is responsible for carrying copper to tissues that need the mineral (42).The cu^{+2} in diet is absorbed from duodenum with aminoacids or small protein,competitively with Fe^{+2} ,Zn and Cd (43)and cu antagonists include sulphide,molybdenum,Zn and Fe .Ceruloplasmin can be inactivated during oxidative stress and Cu can be released leading to excess hydroxyl radical generation,therefore ionic cu is extremely low in the organism(44).It is well known that excessive or insufficient concentration of this element will induce toxicity and deficiency symptoms. Cu^{+2}neutralizes free radicals, or scavenges and may reduce or help to prevent some of the damage they cause (45).

Cytokines are produced in response to microbes and other antigens,different cytokines stimulate diverse response of cells involved in immunity and inflammation(13) .

Tumor necrosis factor alpha and interleukin 1 are key mediator of acute inflammatory reactions to microbes.The occurrence of TNF-α receptors on nearly all cells, TNF-α shows a wide variety of biologic action which might interfere with reproductive functions. Therefore in the current study its concentration was measured in sera and seminal plasma of all studied groups and as described in the material and method section.

The results of Tumor necrosis factor (TNF-α) concentration are listed in Table(3)

The results revealed presence of highly significant increase(p $P<0.001$) in serum concentration of TNF-α in both of the studied groups when compared with that of control group,while the comparison of this concentration in the seminal plasma sample of the oligospermia group showed significant decrease (p $P<0.05$) with the control as well as significant differences(p $P<0.05$)between both of the studied groups were noted. Semen TNF-α levels were negatively correlated with the sperm motility($r=-0.39$, $P>0.05$) in fertile and infertile groups within non significant, meantime has no effect on sperm count and this explain the low level of semen TNF-α of oligspermia group when compared with the control group also explain the non significant change of azoospermia group as this group characterized by a lack of sperm number when compared with the control group. .

The results of TNF in serum agrees with the study of Havrylyuk et al (21) in Poland ,who found significant elevated level of many cytokine in serum samples of infertile males when compared to the control group , meanwhile , the results in seminal plasma were in disagreement only in case of oligospermia group with the findings of many studies such as Kocak *et al* (46) in Turkey who study the relationship between IL-6 and TNF-α with semen parameters in fertile and infertile male and found high level of TNF-α in infertile group when compared with the control. As well as the study of Estrada et al (47) in USA who study the effect of TNF-α on semen parameters and agrees with the slight elevation of azoospermia group in compared with the control group but did not reach statistical significance. .

The association of TNF-α with sperm parameters are in the line with the results presented by Kocak *et al*& Estrada et al (46;47) and disagree with the findings of Qian et al (23) in China who focused on the relationship between IL-17 and other cytokine with semen quality and their result indicated that TNF-α has no effect on sperm characteristics.

Cytokines may interact and antagonize each others action ,and this interaction may also result in synergistic effects.It may act at a distance from the site of production.TNF-α secreted mainely by macrophage and play a critical role in sperm quality due to it can induced sperm membrane lipid peroxidation by increasing ROS releasing within spermatozoa(48). Cytokine elevation may represent a part of a nonspecific acute phase response or may reflect specific interaction between viruses or other stimuli in the immune system

The observed increase in the level of TNF-α in serum patient of the current study may be due to the fact that the TNF-α as a pro inflammatory non specific destruction cytokine and may increase in many non specific condition other than infertility.The elevation of some cytokines present during persistent infection,inflammation in the male genital tract may augment the peroxidation process are directly affect sperm function with a subsequent development of infertility(48).The origin of these cytokines in seminal plasma could be the male genital tract,accessory sex gland and leucocytes ,In addition,cytokine levels In seminal plasma may also be affected by inadequate sample collection and handling (liquefaction,centrifugation) as well as by their molecular heterogeneity and instability(49)

IL-17 is a recently proinflammatory cytokine with strong inflammatory inducing activity, and promotes the development of inflammation, immune response, and other functions(23). Despite the initial discovery over a decade ago, the IL-17 family has not received much attention in the case of infertility (50). However,studies on IL-17 and male reproduction are still lacking. The mechanisms of IL-17 on male fertility need further investigation .

The results of interleukin-17(IL-17) concentration are listed in Table(4)

It is obvious from these results a higher significant decrease(p $P<0.001$) was observed in serum of azoospermia group with the control and highly significant difference(p $P<0.001$) between the two studied groups,while the comparison of this concentration in the seminal plasma samples showed highly significant increase(p $P<0.001$) of both of the studied groups with the control on one hand and significant differences(p $P<0.05$) between the two studied groups on the other hand . Semen IL-17 level is negatively related to sperm motility($r= -0.13$,p $P>0.05$) in fertile and infertile groups but has no significant influence on sperm density.

These results were in a good agreement with Qian *et al* (23) in China who found that semen samples with low activity sperms have higher IL-17 level ,while IL-17 levels have no significant influence on sperm quality,and found that IL-17 level is negatively related to sperm motility. Also the results agreed with EL-Enany *et al* (51) in Cairo who postulated that in chronic testicular infection,Th-17 cells play a key role in the abruption of

testicular function in azoospermia through cytokine mediated effects. As well as agreed with the findings of Sabbaghi *et al* (50) in Iran who study the level of IL-17A in seminal plasma and blood of infertile patients and found that the level of IL-17 was higher in seminal plasma of varicocele patients than the control group, meanwhile disagree with their findings in the serum and their study suggested that the effects of IL-17 on sperm quality are the results of combined actions of a variety cytokines, and the mechanism needs to be further investigated .

It was important to note the gab that observed in the level of IL-17 between seminal plasma and serum of infertile group(oligo&azoospermia) groups and this difference may be due to hypervisocity of seminal plasma which was associated with ROS production,seminal leucocyte concentration and, levels of pro inflammatory cytokines(TNF-α,IL-6) and an anti inflammatory cytokine as well as the influence of infection induced by ROS production on the male accessory glands, furtheremore the shift of the pH between blood and seminal plasma more affected this cytokine level.

The highly significant elevation of the seminal IL-17 concentration for oligospermia and azoospermia groups when compared with that of control may be attributed to many causes as the results revealed inverse relationship between sperm motility and seminal plasma IL-17 level could be explained as the sperm motility increase the level of IL-17 decrease and vise versa. The elevation in the concentration of IL-17A can cause the increase in the level of inducible nitric oxide synthase-2 and nitric oxide(NO) production , as the NO short-lived free radical which is involved in many pathologic and physiologic function of spermatozoa(52). Therefore,the elevation of IL-17 concentration may affect the spermatozoa production,motility and morphology in the patients *via* the increase in the level of nitric oxide(53).

Although specific actions of IL-17 on testicular cells are still undervaluated,this cytokine acts through recruitment,activation and migration of a broad range of inflammatory cells increasing the expression and action of proinflammatory cytokines,metalloproteinases,and chemokines(54).Consequently ,over expression of IL-17 could not only substantially impair blood-testis barrier but also probably adversely affects germ cells and normal spermatogenesis,and in turn could ultimately lead to azoospermia.Over expression of IL-17 that occurs during inflammation might participate in inducing azoospermia through cytokine activation of macrophages and cytotoxic lymphocyte leading to germ cell apoptosis by the perforin-granzyme or the fas-fas ligand pathways(55).

Interleukin-10 (IL10) is a multifunctional anti-inflammatory cytokine that is produced by various cells including monocytes, macrophages, B cells, T cells and mast cells(56). IL10 in return modulates the performance of these various cells with important consequences to their ability to activate and sustain immune and inflammatory responses(57). The role of IL10 plays in the immune response an inhibiting the production of various pro-inflammatory cytokines produced by a large number of different cells(56).

Table(5) shows IL-10 concentration in sera and semen of all the studied groups. The results revealed that IL-10 levels in both of the studied groups(oligo & azoospermia) in serum were higher than that of the control group and this difference was statistically insignificant($p>0.05$).While their results in seminal plasma showed significant decrease($P<0.05$) with the control group. No significant correlation was recorded between IL-10 concentration and sperm parameters.

The results in serum were in a good agreement with Havrylyuk et al (21) who found high concentration of IL-10 in blood serum samples of idiopathically infertile males comparing to the control group. .

The high level of IL-10 may be due to the peroxidation processes are directly affect sperm functions by secreting IL-10 which secreted due to inflammatory processes.Mean while the results in seminal plasma were agreed with many of studies such as Camejo(58) in Venezuela who found lower levels of IL-10 in seminal plasma of infertile men than that of fertile men and hypothesis that the decreas in suppressive activity might be due to a decrease of immunosuppressive molecules or an increase of pro inflammatory one. Also the results were agreed with Huleihel *et al* (20) in Israel who identified the immune factors involved in the inhibitory effect of seminal plasma from fertile and infertile men and found that the level of IL-10 were significantly decreased in seminal plasma of infertile as compared to the fertile men. As well as the results were in agreement with Castiglione et al(59) who found seminal IL-10 levels in prostatitis vesiculitis and chronic bacterial prostatitis patients were lower than those found in the control.

Meanwhile disagree with data reported by Rajasekaran *et al* (60) who observed no significant differences compared to seminal plasma from fertile and infertile men.

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Table (4) Mean Values of conc.of inter leukin 17 concentration in sera and seminal plasma samples of control and patients groups .

Groups	Samples N0.	Age(years) (Mean±SD)	mean±SD IL-17 (ng/l) Serum	Seminal plasma
Control	25	29.4±4.12 range (20-48)	30.56±11.06	26.76±11.16
Oligospermia	20	35.2±6.90 range(22-46)	**b 0.60±11.18 P.value 0.9 a 0.000 b	b*** a 76.20±28.5 p.value 0.000 a 0.004 b
Azoospermia	20	31.3±7.52 range (20_50)	**a 16.50±4.88 P.value 0.000 a	b* ** a 111.85±30.19 p.value 0.000 a

a** high Significant difference in comparison to control at ($p\leq0.001$).

b** high Significant difference in comparison between tow patients groups at ($p\leq0.001$).

Table (5) Mean Values of interleukin 10 concentration in sera and seminal plasma samples of control and patients groups .

Groups	Sampls No.	Age (years) mean±SD	mean±SD IL-10 pg/ml Serum	Seminal plasma
Control	15	29.4±4.12 range (20-48)	243.2±31.65	357.13±87.9
Oligospermia	15	35.2±6.90 range(22-46)	279.7±52.2 p.value 0.051 a 0.9 b	a * 289.6±45.7 p.value 0.008 a 0.43 b
Azoospermia	15	31.3±7.52 range (20_50)	277.6±50.8 p.value 0.0563 a	a* 307.87±45. 9 p.value 0.04 a

a* Significant difference in comparison to control at ($p\leq0.05$)

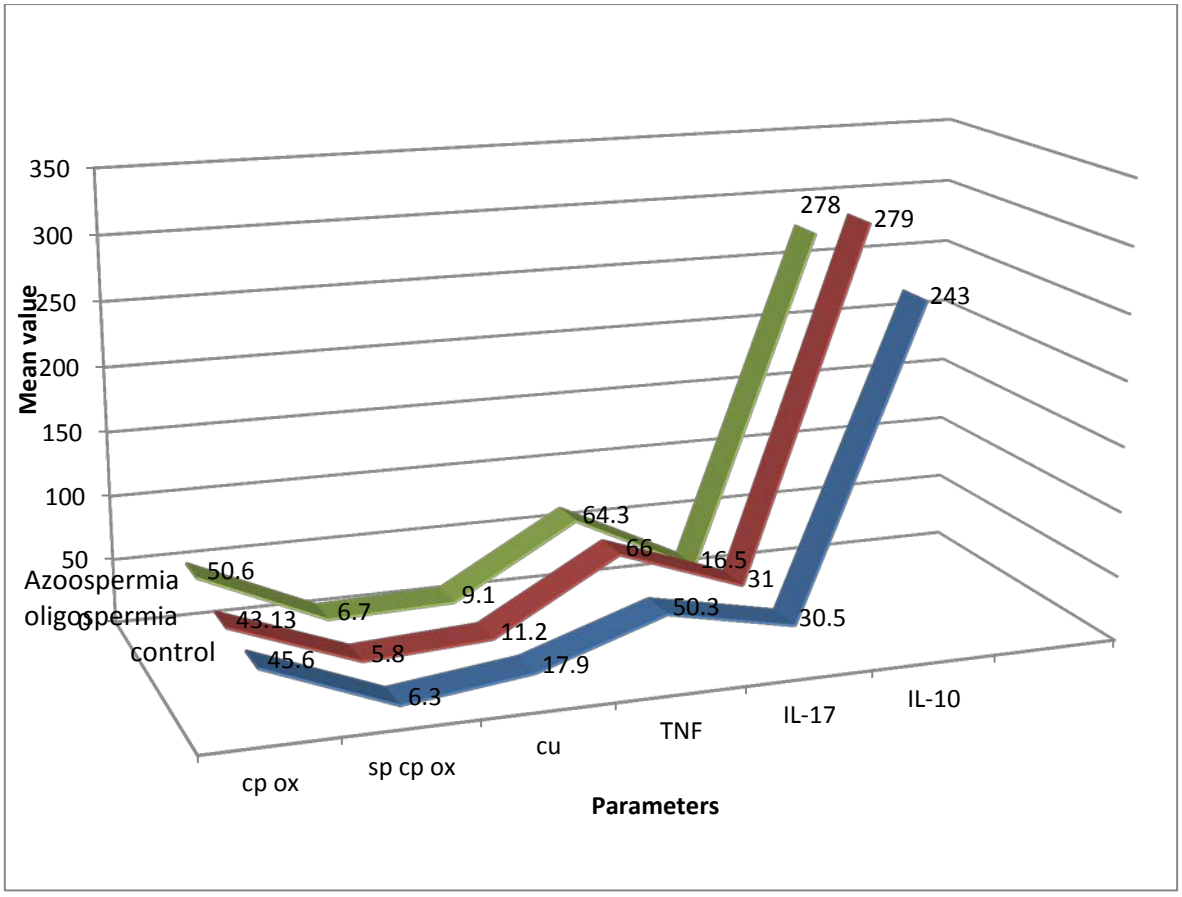

Figure 1 All average values of biochemical and cytokines parameters in the serum of fertile (control) and infertile (oligo,azoospermia)groups .

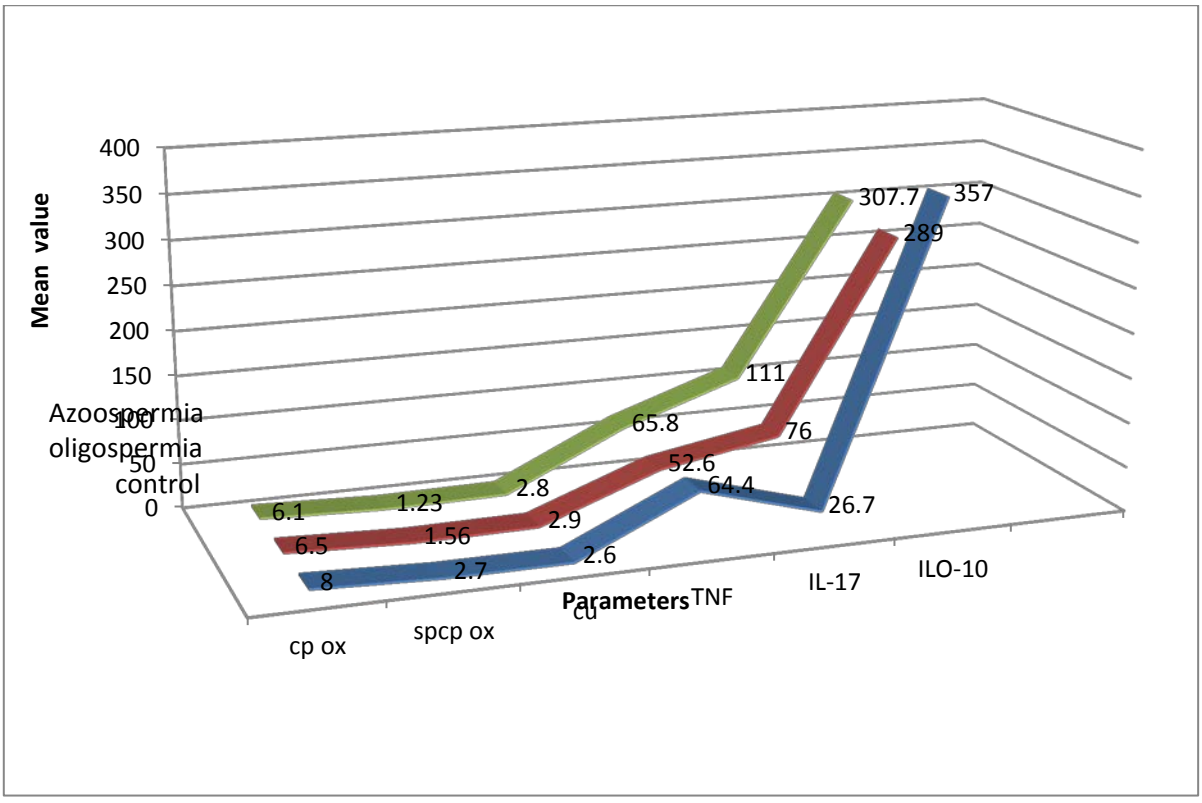

Figure 2 Average values of the biochemical and cytokine levels in seminal plasma of fertile and infertile groups.

Decrease in IL-10 levels was observed in the seminal plasma of infertile male may be due to the presence of active proteases or to the presence of other immunoregulatory factors or soluble cytokine receptors which may regulate the capacity of IL-10 production by producer cells in the genital tract(20). Also the immune cells such as NK,Ts,TH_1 and TH_2 cells in seminal plasma of the study group may affect IL-10 levels in seminal plasma ,which act as the suppressor cytokine of the immune response in the seminal plasma of infertile male with infection(60) . Several studies mention that different cytokines have no correlation with semen parameters(21) which disagree with the findings of the present study. Meanwhile the positive effects of cytokines in the male reproductive function can be summarized as follows IL-8 have been reported to play multifunctional interactions in the testis. The proinflammatory cytokins are considered as a paracrine defense factors in the male gonad whereas the soluble immunosuppressive factors are the anti-inflammatory cytokines that protect testis against autoimmune reaction in cooperation with anatomical blood testis barrier(61,62). Through cytokine research within the male reproductive system Fraczek *et al* (63) indicated several aspects about the presence of variety of cytokines in human seminal plasma and differences in cytokine levels between fertile and infertile men, also negative correlation between semen parameters and some cytokine levels as well as,usefulness of some cytokines as clinical markers of male infertility subtypes(63).

Table 6 : Personal correlation between serum and seminal plasma of all the parameters.

Serum Seminal plasma l	Control	oligospermia	Azoospermia
Cp oxidase	-0.413*		
SP cp oxidase	-0.042*		
TNF-α			0.508*

*Correlation is significant at the 0.05 level., - negative correlation

Table 7: Personal correlation between all the parameters of all the studied groups .

Association between	Pearson correlation (r)	P value
TNF-α and cp oxidase in serum of control group.	0.650	0.001**
IL-17 and TNF-α in serum of oligospermia group.	0.650	0.002*
IL-17 and sp cp oxidase in serum of control group	-0.493	0.022*
IL-10 and cp oxidase in serum of azoospermia group	-0.72	0.003*
IL-10and sp cp oxidase in serum of azoospermia group.	-0.543	0.036*
IL-10 and IL-17 in serum of control group.	0.723	0.005*
IL-10 and IL-17 in seminal plasma of oligospermia group.	0.650	0.011 *
IL-10 and cu in seminal plasma of azoospermia group .	-0.647	0.023 *

**Correlation is a highly significant at the 0.001 level.,
– negative correlation
*Correlation is significant at the 0.05 level.

The average values of the biochemical and cytokines levels in serum and seminal plasma have been shown in Figure 1 and 2 respectively.

Among the infertile groups,azoospermia group ,the levels of cp oxidase and sp cp oxidase are significantly high compared to other groups,meanwhile the levels of cu and IL-17 are lower than the other groups,while oligospermia group showed high levels of TNF-α and IL-10 when compared to other groups.

In seminal plasma the level of cp oxidase and its specific activity and IL-10 were significantly higher in fertile control group than the other groups while marked significant elevation of IL-17 was recorded in infertile(azoospermia) group when compared with the other groups(Figure 2).

The personal correlation was applied to assess the relationship among studied parameters and to check the possibility of using semen as a sample of analysis instead of serum .Table 6 showed the significant correlation between serum and seminal plasma of all the parameters(data not shown for non significant correlation), meanwhile Table 7 showed the significant correlation among all parameters included in the present study. .

CONCLUSIONS:

The data obtained revealed that the study of such parameters in blood plasma can not be used as a indicator of what happening in seminal plasma, excepted for Cp oxidasee and its specific activity of control group and tumor necrosis factor alpha of azoospermia group .Meanwhile the present study has demonstrated significant changes in immunological parameters studied in groups of infertile patients.Furthermore, Cp oxidase&specific activity, TNF-α and IL-17 were negatively correlated with sperm motility within non-significant value.

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